Malaysian Journal of Science **19**: 35-40 (2000)

Natural and semisynthetic substances with antitubulin activity

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ABSTRACT The tubulin test, a powerful tool to detect anticancer agents, was outlined briefly. Three classes of compounds, defined by their sites of fixation and their mechanisms of action were discussed.

ABSTRAK Ujian tubulin, satu kaedah yang efektif untuk pengesanan agen antibarah, diterangkan secara ringkas. Tiga jenis kumpulan sebatian dikelaskan mengikut tapak aktif dan mekanisme tindakan dibincangkan.

(Tubulin test, anticancer, lignan)

INTRODUCTION

Today, cancer remains one of the main causes of mortality in the world and chemotherapy is one of the foremost means of ceasing or decreasing the progress of this illness. Most of the anticancer agents are of natural origin: plants or marine organisms. In the pursuit of discovering new anticancer drugs, acquiring a good tool to reveal the anti-tumour activity of a given plant is of prime importance and for a long time, the inhibition of cultured cells (KB cells) is the most popular method.

An anticancer compound must kill the cell or stop its development. To do that, it can act at different stages of the cell cycle. Many of the cell cycle inhibitors do not act specifically at a given stage of this cycle, for instance the alkylating agents can operate on a lot of targets and at any moment. Some others inhibitors are more specific like the inhibitors of the enzymes associated with DNA, the inhibitors of the topoisomerases I and II belong to this category. However, compounds interacting at a very short domain of the cell cycle like mitosis would be more specific which is the case of the spindle poisons like colchicine, vinblastine or taxol that interfere with the main component of this spindle: the tubulin-microtubule system. Unfortunately, this system is not only present during mitosis but it is also involved in several cellular activities.

This paper describes briefly the tubulin-microtubules system and its application to a simple test. The tubulin-microtubules system has been discovered in the sixties by the research on the cellular receptor of colchicine, a well-known agent in inducing abnormal mitosis and later, thanks to the action of this poison, it has been proven that tubulin was involved in two kinds of activity: the transport in the cell (axonal transport, cellular secretion, mitosis) and a constitutive role (in the cytoskeleton and in cilia and flagella) [1].

MATERIALS AND METHOD

Tubulin can be easily prepared from mammal brain where it represents about 20% of the soluble proteins. After several cycles of temperature-dependent assembly-disassembly process, an 80% pure protein is obtained [2]. Purification can be achieved by anion exchange chromatography. Tubuline can be kept at -20° for 2-3 months or several years at -200°C.

The active form of this system is the equilibrium between tubulin and microtubule. *In vivo*, the regulation of this equilibrium is largely unknown but *in vitro* some parameters are essential: temperature (0° and 37°C), magnesium or calcium ions, Guanosime Tri-Phosphate and other proteins called Micotubule-Associated-Proteins. Tubulin is a heterodimer of 100 000 kDa. It is an acidic and hydrophobic protein with a highly conserved sequence; the microtubule form is a cylinder constituted of dimers without any covalent bond between them.

The behavior of this protein could be followed by several means: electronic microscopy, light

diffusion, viscosity but the simplest method is the measure of the optical density of a tubulin solution in a U.V. spectrophotometer (Fig. 1). The formation of microtubules is observed at 37°C by an increase of the optical density at 350 nm and the depolymerization process at 0°C. If an inhibitor is added, the speed of the assembly or disassembly is modified [2]. Then by recording the effects at different concentrations, an IC_{50} of the drug can be determined and compared with those of an already known poison of the same structural class, measured the same day on the same tubulin sample.

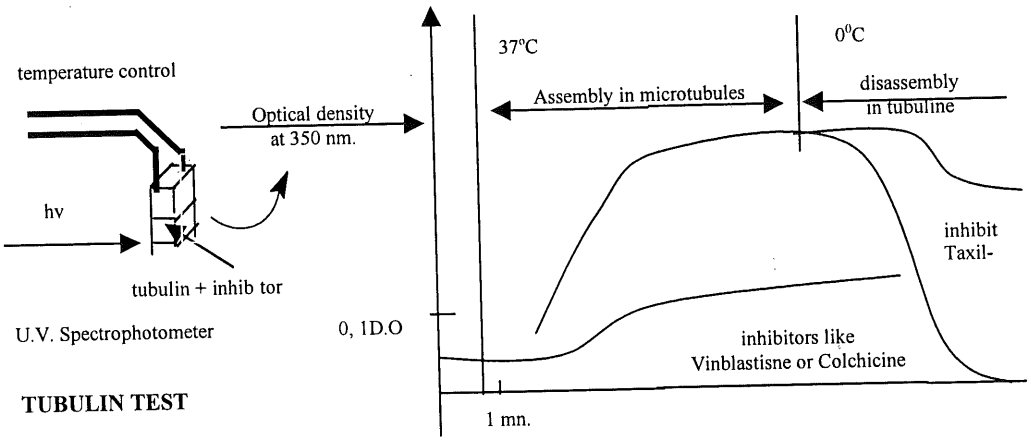


Figure 1

RESULTS AND DISCUSSION

Among the tubulin inhibitors, three classes of compounds can be defined according to their site of fixation and their mechanism of action. These sites are the cochicine site, the vinblastine site and the taxol site.

Compounds inhibiting at the cochicine site are represented by a family of natural products called lignans (colchicine, steganacine, podophyllotoxine, Fig. 2). They possess two aromatic rings. These substances inhibit the assembly of tubulin by a temperature-dependent fixation on one site; sometimes this binding is nearly irreversible [3,4,5]. A lot of other structurally unrelated drugs, bind tubulin at this site. However, there is no anticancer compound used from this class at present.

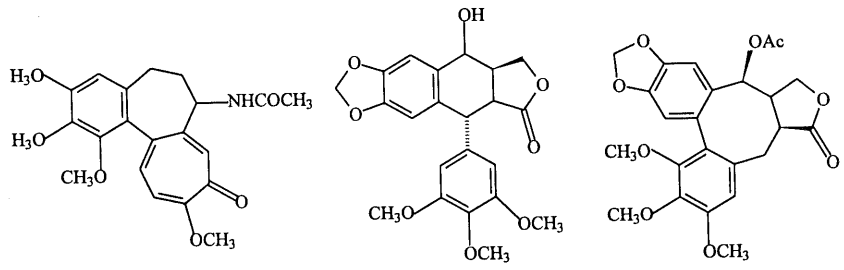
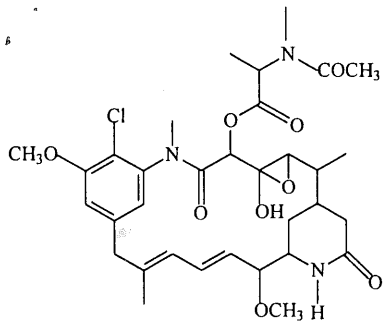
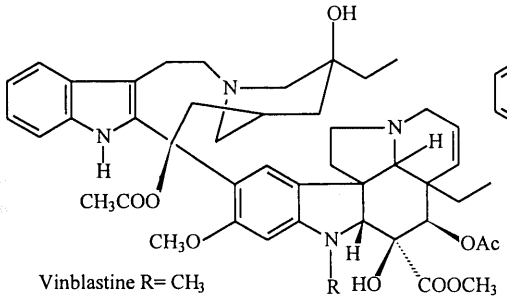


Figure 2

The vinblastine site class of compounds are represented by vinblastine and vincristine which are also inhibitors of the assembly process. They possess two sites of fixation responsible for the inhibition of assembly and for a false polymerization called "spiralization" of tubulin [6,7] . The latter effect led to the selection of navelbine as a new anticancer agent [8]. Actually, navelbine was only a secondary product in the vinblastine synthesis, but its particular behavior on tubulin led to its biological study. All these compounds are used in chemotherapy. Moreover, some other unrelated structures interact at the same site on tubulin: maytansine or dolastatine [9] (Fig. 3).

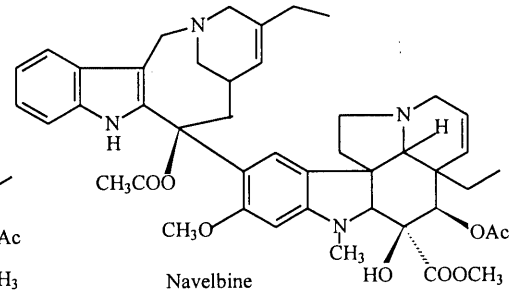


Maytansine

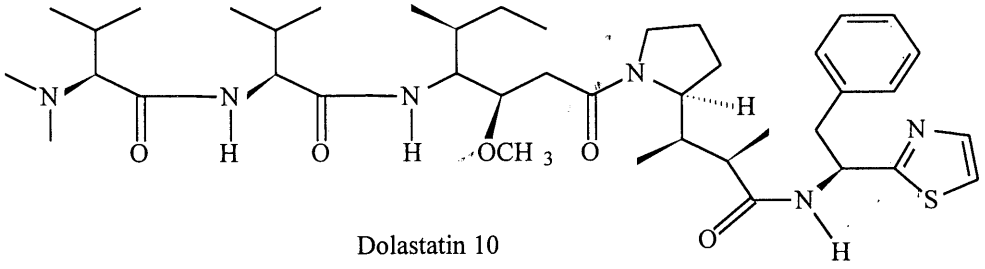


Vinblastine R= CH_3

Vincristine R = H



Navelbine



Dolastatin 10

Figure 3

Taxol, found in the bark of the yew tree [10], is an inhibitor of the disassembly process and a promoter for the assembly at 0°C in the absence of GTP [11]. It represents the taxol (paclitaxel) site class of compounds.

There is only one site of inhibition on the tubulin and the fixation occurs only on the microtubule. 10-deacetylbaccatin III, a natural chemical precursor of taxol, and docetaxel (taxotere®), an intermediate in the taxol synthesis, were found more active than taxol on tubulin [12]. These two compounds are now used in the treatment of ovary and breast cancers. Only one compound that is not structurally related to taxol, epothilone, seems to interact with tubulin at the same site [13] (Fig. 4).

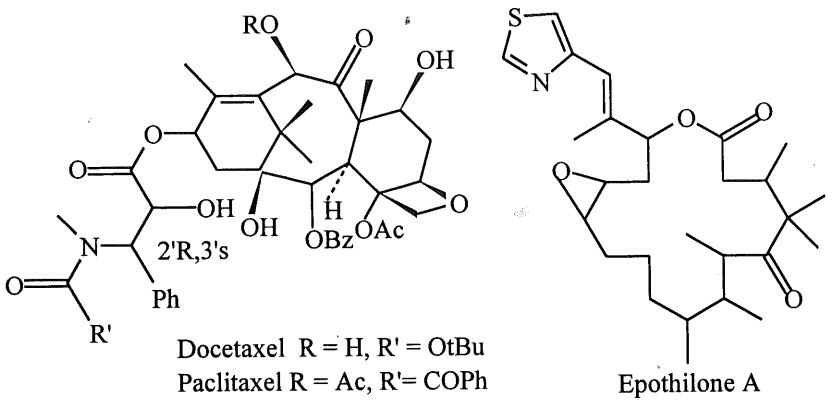


Figure 4

Unfortunately, there are limits to the use of this test. For example, it has been mentioned that ellipticine or reserpine binds tubulin at the colchicine site; the level of this activity is not high enough to have a biological significance. On the other hand, tubulin test alone is not sufficient to evaluate the anticancer potency of any drug. For instance, the etoposides, VP-16 and VM-26 (Fig. 5), structures of which are very similar to podophyllotoxine, do not show any effect on tubulin. However, they are active on the topoisomerase enzymes.

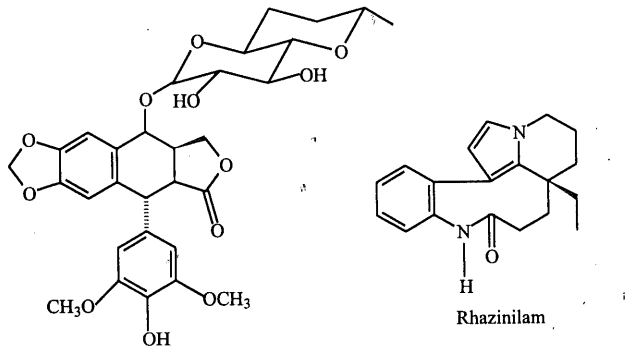


Figure 5

The tubulin test can also be used to detect the presence of antimitotic compounds in plant extracts [14]. An ethanolic extract of *Kopsia singapurensis* was found active on tubulin polymerization but further studies showed that the active substance was an artefact produced in the extract in presence of oxygen and biochemical experiments demonstrated that its action was more complex because, not only its behavior was similar to those of colchicine, but it provokes a spiralization of tubuline like vinblastine and inhibits the cold-induced disassembly like taxol [15]. It seems that this drug belongs to a new class of antitubulin agents with an overlapping with the vinblastine family.

Many analogues of this molecule have been synthesized in order to increase the affinity of these compounds for tubulin but all the modifications decreased the activity. Rhazinilame, like colchicine analogues, also belongs to the family of biaromatic compounds and it seems that this substructure is often recognized by different sites on tubulin. Fig. 6 shows more examples of antitubulin compounds. They are interesting in the

topological study of the tubulin sites. Belonging to this class, the two last substances, a flavonol (I) and a chalcone (II) [16], were discovered by systematic assays on tubulin of new plant extracts; they could be considered as other model compounds for the research of antimitotic drugs.

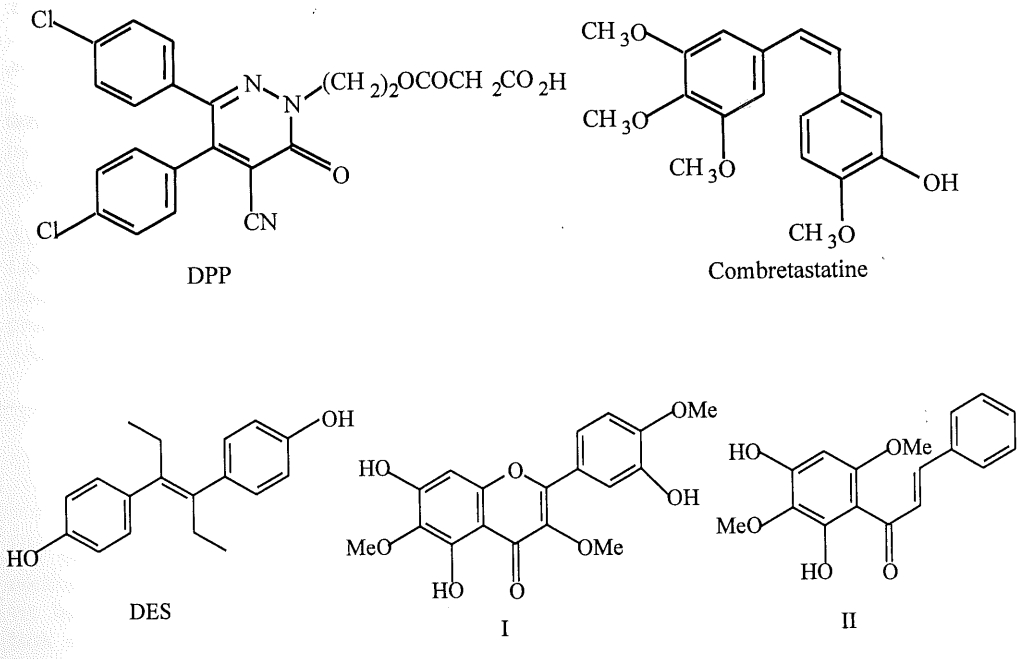


Figure 6

CONCLUSION

The "tubulin test" is a very useful assay for the discovery of new anticancer agents as it has been shown with Navelbine and Taxotere. It shows a high efficiency among the compounds of a same active family, allowing the establishment of the structure activity relationships in this series. It must be outlined, however, that an interaction with tubulin is necessary but not a sufficient condition for an *in vivo* activity. The level of this activity must be high enough in order to be significant and consequently, have a specificity of action in the cell.

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